Fibroblast growth factor 21 induces lipolysis more efficiently than it suppresses lipogenesis in goat adipocytes

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Received: 18 July 2017 / Accepted: 17 July 2018 / Published online: 26 July 2018
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Abstract Fibroblast growth factor 21 (FGF21) potentially regulates glucose and lipid metabolism in energy homeostasis. We investigated dynamic changes in goat adipocytes treated with 75 nM FGF21 for 24, 36 and 48 h. Compared to controls, FGF21-treated adipocytes displayed smaller lipid droplets and altered levels of the mRNA transcripts encoding several lipolysis genes. The genes with elevated mRNA levels included: *ATGL*, *HSL*, *CPT-1*, and *UCP1*, and this was observed mainly at 24 and 36 h ($P < 0.05$). Some gene expression was attenuated including lipogenesis genes, such as *SREBP1*,

PPAR γ , *C/EBP α* , and *ACC*. This attenuation was observed mainly at 24 h ($P < 0.05$). Among the genes that were significantly induced or inhibited, *ATGL*, *PGC1 α* , and *C/EBP α* were observed a significant effect at 48 h ($P < 0.05$). In addition, FGF21 treatment greatly increased number of mitochondria and the expression of genes implicated in mitochondrial biogenesis, such as *PGC1 α* , *NRF1*, and *TFAM*. These results suggest that FGF21 treatment induced lipolysis more effectively than it suppressed lipogenesis in goat adipocytes, and that mitochondrial biogenesis plays an important role in these cells.

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Graphical abstract

Keywords FGF21 · Adipocyte · Lipolysis · Lipogenesis · Mitochondria

Abbreviations

FGF21	Fibroblast growth factor
ATGL	Adipose triglyceride lipase
HSL	Hormone sensitive lipase
CPT-1	Carnitine palmitoyltransferase 1
ACC	Acetyl-coa carboxylase
FAS	Fatty acid synthase
SREBP1	Sterol regulatory element binding protein 1
C/EBPα	CCAAT/enhancer binding protein
PPARγ	Peroxisome proliferator-activated receptor γ
PGC1α	Peroxisome proliferator activated receptor-γ co-activator 1-α
NRF1	Nuclear respiratory factors 1
TFAM	Mitochondria transcription factor A
UCP1	Uncoupling protein 1
GCG	Glucagon gene
COX2	Cytochrome c oxidase 2
HSP-90	Heat shock protein 90
ALAS	Aminolevulinate synthase

HMBS	Hydroxyl methyl bilane synthase
FIS1	Fission protein 1
OPA1	Optic atrophy 1
Mfn1	Mitochondria fusion protein 1
Mfn2	Mitochondria fusion protein 2

Introduction

Adipose tissue is the major storage site of triglycerides (TG), an important fuel source providing free fatty acids (FFA) and gluconeogenic carbons in the form of glycerol. With escalating obesity and diabetes in current populations the adipose tissue in dietary sources is a concern (Lasa et al. 2012). Compared to other livestock such as pigs, cattle, and sheep, goats deposit less subcutaneous and intramuscular fat (Webb et al. 2005). Hence, goat meat appeals to clients as a source of lean meat, especially in developed countries with a high incidence of cardiovascular disease. Therefore, insights into the molecular machinery of goat lipid metabolism may contribute to our understanding of fat-related diseases and meat quality of domestic animals.

Fibroblast growth factor 21 (FGF21) specifically regulates glucose and lipid metabolism (Coskun et al. 2008; Emanuelli et al. 2014), and therefore plays critical role in energy homeostasis in mammals (Kharitonov et al. 2005). FGF21 is predominantly enriched in energy regulating organs like skeletal muscle and adipose tissue (Kharitonov and Larsen 2011; Murata et al. 2011). Increasing *FGF21* expression elevates lipolysis in adipose tissue and plasma levels of FFAs during fasting (Hotta et al. 2009). Mild obesity and adipocyte hypertrophy were discovered in KO-FGF21 mice (Badman et al. 2009; Hotta et al. 2009). Many metabolic disorders in rodents and nonhuman primates have been cured through recombinant FGF21 treatment (Xu et al. 2009). Additionally, FGF21 might be closely related to mitochondria function (Luo and McKeehan 2013), since skeletal muscle and brown adipose tissue are rich in mitochondria (Fisher et al. 2012; Ji et al. 2015).

Although studies on FGF21 have been carried out in model mammals such as human and mice, the biological function of the *FGF21* gene in domestic animals is still limited. Here, we systematically illustrate the effects of FGF21 on the size of lipid

droplets, gene expression in lipid metabolism (lipolysis and lipogenesis), and number of mitochondria within goat adipocytes. These results provide useful data on lipid metabolism in large-size mammals.

Materials and methods

Isolation of preadipocytes from fat tissue

Three healthy male Nanjiang Brown kids (3d old) were provided by the Nanjiang Brown Goat Breeding Center (Nanjiang, Sichuan, China). Subcutaneous adipose tissue was collected from beneath back skin and rinsed with phosphate buffered saline (PBS medium supplemented with 50 U/mL penicillin and streptomycin; Hyclone, Logan, UT, USA). Tissue was then sliced into approximately 1-mm³ sections under sterile conditions and digested with type I collagenase for about 60–90 min at 37 °C with constant agitation. The digested tissue was filtered through a 200 µm nylon mesh screen to separate cells from undigested tissue fragments and debris and then centrifuged at 1400 ×g for 5 min. The cells were centrifuged 1400 ×g for 5 min, counted using a hemocytometer, resuspended in DMEM-F12 medium (Hyclone) supplemented with 10% (v/v) fetal bovine serum (Gibco, Mulgrave, VIC, Australia).

Preadipocyte culturing

In order to normalize the growth of goat preadipocytes, we first studied the differentiation of goat adipocytes. Preadipocytes were cultured under humidified air containing 5% CO₂ at 37 °C. The medium was changed every second day. The cells became confluent after 48 h (day 2), differentiation into adipocytes was initiated as follows: medium was supplemented with 0.5 mM 3-isobutyl-methylxanthine (MIX; Sigma St. Louis, MO, USA), 1 µM dexamethasone (Sigma), and 10 µg/mL insulin (Sigma) for 2 days. Then the culture medium was replaced with DMEM-F12 supplemented with 10% fetal bovine serum and 10 µg/mL insulin for another 2 days. Thereafter, the cells were cultured in DMEM-F12 medium containing only 10% FBS.

On day 4, the adipocytes harbored a few obvious lipid droplets (Fig. 1a), and on day 8 a large number and larger sized lipid droplets arose (Fig. 1b). On day 12 approximately 90% of the adipocytes differentiated

(Fig. 1c). These results imply that after about 12 days of culture, the preadipocytes matured into adipocytes.

FGF21 treatment and cell harvesting

To achieve optimal experimental conditions, we based our protocol on previous studies involving preadipocyte culture as well as research on FGF21 that was reported in human cells (Fisher et al. 2012). We added recombinant human FGF21 protein (PROSPEC, Rehovot, Israel) to mature adipocytes (day 12) with final concentrations of 0 nM, 50 nM, 75 nM and 100 nM and then harvested cells 24 h later. The mRNA levels of *PGC1α*, *PRDM16*, and *UCP1* increased in adipocytes treated with FGF21 of 75 nM (Fig. 1d).

Therefore, in further studies, goat adipocytes cultured 12 days were treated with FGF21 at final concentration of 75 nM. The adipocytes were harvested to extract RNA and DNA or analyzed histochemically, at 24, 36 and 48 h.

RNA and DNA extraction and cDNA synthesis

Total RNA (TRIzol reagent, Invitrogen, Carlsbad CA, USA) and genomic DNA (TIANGEN, Beijing, China) were extracted using the manufacturer's protocol. Their concentration and quality were further assessed using the NanoDrop spectrophotometer (Bio-Rad, Benicia, CA, USA). cDNA was synthesized from 1 µg of total RNA using the PrimeScript RT reagent Kit with oligo-dT primers (Takara, Dalian, China).

Lipid droplet staining

Cytoplasmic lipid droplets were stained using oil red O (Sigma). Generally, adipocytes were fixed with 4% formalin in phosphate buffer for 1 h at room temperature and stained for 30 min with a filtered 60% (w/v) oil red O solution in distilled water. Cells were washed with distilled water and then visualized and photographed using light microscopy.

The cytoplasmic lipid droplets were analyzed using a phase-contrast microscope and Image-Pro Plus software from Media Cybernetics (Carlsbad, CA, USA). Stained adipocytes were scanned using × 40 objective magnifications. A field at objective magnification was chosen for analysis.

Fig. 1 Morphologic characteristics of goat preadipocytes: Goat preadipocytes were stained with oil red O solution, visualized by light microscopy, and photographed. Preadipocytes were stained with oil red O after differentiation on day 4 **a** and day 8 **b**. **c** Preadipocytes differentiated into adipocytes contained

lipid droplets in the cytoplasm. Adipocytes were stained with oil red O. **d** The gene expression of *UCP1*, *PRDM16* and *PGC1α* were detected by qRT-PCR (SEM, $n = 3$, * $p < 0.05$). (Color figure online)

Fluorescent mitochondrial visualization

MitoTracker Green FM (Invitrogen, Carlsbad CA, USA) was used to evaluate mitochondrial number in adipocytes. The cells were incubated with 150 nM MitoTracker at 37 °C for 30 min and washed 3 times with prewarmed PBS. Mitochondria were quantified using fluorescence microscopy and Image-Pro Plus software from Media Cybernetics (Carlsbad, CA, USA).

Cloning of goat FGF21 CDS

The primers for goat *FGF21* were designed according to its predicted cDNA sequence (GenBank accession no.XM_005692688.1) (Table 1) and synthesized by the Tsing Ke Biotechnology Company (Chengdu, China). PCR was performed using 12.5 μ L 2 $\times$ Taq PCR master mix (TIANGEN, Beijing, China), 1 μ L each of forward and reverse primers (final 10 μ M), 2 μ L cDNA and 8.5 μ L sterile water. Conditions for PCR were 5 min at 95 °C, followed by 35 cycles of denaturation at 94 °C for 30 s, 67 °C (annealing temperature) for 30 s, and 72 °C for 1 min, and final extension at 72 °C for 7 min. The PCR products were purified, cloned (pMD 19-T vector, Takara, Dalian,

China), and then sequenced by Tsing Ke Biotechnology Company (Chengdu, China).

Quantifying the mRNA levels of target genes

The qRT-PCR was performed with a total volume of 10 μ L containing 5 μ L 2 $\times$ SYBR Premix ExTaqII, 0.4 μ L each primer (10 μ M) and 0.8 μ L diluted cDNA. The PCR conditions were as follows: 95 °C for 30 s; 40 cycles of 94 °C for 15 s, primer specific T_m for 30 s, and 72 °C for 30 s. *HSP-90*, *ALAS* and *HMBS* were used as internal control genes (Najafpanah et al. 2013). Primer sequences were listed in Table 1.

Investigating mitochondrial DNA copy number

The copy number of mitochondrial DNA (mtDNA) per adipocyte was assessed using real-time qPCR. The amount of COX2 and GCG, single copy gene in mitochondrial and nuclear DNA, respectively, was analyzed. PCR conditions were similar to qPCR described previously except using DNA as template. The ratio of COX2 to GCG was used to quantify mtDNA molecules in each adipocyte.

Table 1 Specific primers used in this study

Gene	Sequences 5' to 3'
<i>FGF21</i>	Forward ^a : ATGGGCTGGGACGAGGCCAAGTTCAAG Reverse: TCAAGAAGTGTAAGTGGGGCTTCGG
<i>FGF21</i>	Forward: ACTGTGGGTCCCTGTGCTG Reverse: GGGCTGGAGTCTGGAATGG
<i>ATGL</i>	Forward: GGAGCTTATCCAGGCCAATG Reverse: TGCGGGCAGATGTCACTCT
<i>HSL</i>	Forward: GCCAACACCCAGGAAGAAA Reverse: GAATGCCCCGAAAGACG
<i>ACC</i>	Forward: AAGGTTATGTGAAGGATGTGGATGA Reverse: CAATAATCTTCTGATGCCTGCGTT
<i>FAS</i>	Forward: GGGCTCCACCACCGTGTTCCTCA Reverse: GCTCTGCTGGGCCTGCAGCTG
<i>SREBP1</i>	Forward: CTGCTGACCGACATAGAAGACAT Reverse: GTAGGGCGGGTCAAACAGG
<i>C/EBPα</i>	Forward: CCGTGGACAAGAACAGCAA Reverse: GGCGGTCAATTGTCACTGG
<i>PPARγ</i>	Forward: GTGTCACTCCTGAACGAAAT Reverse: GGAAATGCTGGAGAAGTCAA
<i>PGC1α</i>	Forward: AAGCCAACCAAGATAACC Reverse: TACAACCTCAGACTGCTCGGG
<i>FIS1</i>	Forward: ACAGAGCCGCAGAACAAAC Reverse: CACAGCAAGTCCGATGAGT
<i>OPA1</i>	Forward: AGGGTTGTTGTGTTTGG Reverse: CCGCATTCGGAGTTCTA
<i>Mfn1</i>	Forward: CGGGCACAGATGTCACTA Reverse: GGCTTGGAAGTCGCTCA
<i>Mfn2</i>	Forward: CGTGGTCCTCAAGGTTTACA Reverse: TCCATTTGACTCCGCACA
<i>NRF1</i>	Forward: TCCAAACCCAACCCTGTC Reverse: GGGATAAATGCCCGAAGC
<i>TFAM</i>	Forward: TGGCACATCACAGGTAAA Reverse: GTTCCTCCCAGGATTTC
<i>UCP1</i>	Forward: ATCTCCACGGTCCCAAAC Reverse: ACAACAGCGGACACGAAG
<i>GCG</i>	Forward: GCTGCTGCTTTGTCTGC Reverse: GCTTCGGGTTCTGGATT
<i>COX2</i>	Forward: GTAGAAACGGTCTGAACT Reverse: TTTACTGTGAGGGATGG
<i>HSP-90</i>	Forward: GCCCGAGATAGAAGACGTTG Reverse: AGTCGTTGGTCAGGCTCTTG
<i>ALAS</i>	Forward: ATGTGGCCCACGAGTTTGG Reverse: CTTGTGCTGGCGATGTACC
<i>HMBS</i>	Forward: CTTGCCAGAGAAGAGTGTGG Reverse: CAGCCGTGTGTTGAGGTTTC

^aThe primer used for real-time PCR of *FGF21* cloning and other primers used for qRT-PCR

Statistical analyses

The expression levels of target genes were calculated using $2^{-\Delta\Delta C_t}$ methods (Livak and Schmittgen 2001). Values between treatment and control group at each time point were compared via unpaired *t* test in IBM SPSS version 22. Data are presented as mean $\pm$ SE. $P < 0.05$ was considered significant.

Results

Characteristics of goat FGF21

The goat *FGF21* CDS sequenced here was the same as the predicted nucleotide sequence of FGF21 in the NCBI database (XM_005692688.1). It contained an open reading frame (ORF) of 630 base pairs and encoded 209 amino acids. The putative goat FGF21 protein shared 99.04, 66.53, 75.24, 91.39 and 85.71% identity with that of cow (XP_010813507.1), sheep (XP_011950505.2), mouse (AAH49592.1), pig (ACS34770.1), and human (AAH18404.1), respectively.

In addition, goat and human FGF domains (126 residues from Gln⁴³ to Pro¹⁶⁸) were highly conserved, retaining 86.51% identity. They had almost the same receptor interaction binding sites (a conserved 17 residue segment) and similar heparin binding sites (glycine box) (Fig. 2). Therefore, it is reasonable to use human FGF21 in this study.

FGF21 decreased the size of lipid droplets in goat adipocytes

Generally, adipocytes displayed significantly smaller lipid droplets following FGF21 treatment (Fig. 3a).

24 h after FGF21 treatment, small lipid droplets ($< 50 \mu\text{m}^2$) increased by 8% (FGF21 treatment, 33.07%, $n = 309$; control, 25.57%, $n = 257$; $P = 0.026$), while medium ($50\text{--}99.9 \mu\text{m}^2$) and large ($> 100 \mu\text{m}^2$) droplets decreased by 3% (FGF21 treatment, 32.29%, $n = 316$; control, 35.27%, $n = 275$; $P = 0.044$) and 5% (FGF21 treatment, 34.63%, $n = 503$; control, 39.16%, $n = 453$; $P = 0.074$), respectively. At 36 h the percentage of large lipid droplets increased slightly, accompanied by a slight decrease of small droplets. Furthermore, the percentage of both medium and large size lipid droplets increased whereas small droplets declined dramatically at 48 h after FGF21 treatment (Fig. 3b).

FGF21 inhibited lipogenesis and simultaneously stimulated lipolysis in goat adipocytes

Lipid amount demonstrates the dynamic equilibrium of fat deposit and expenditure. Obviously, FGF21 treatment dramatically suppressed the expression of *SREBP1*, *PPAR γ* , *C/EBP α* and *ACC* at 24 h (Fig. 3c; $P < 0.05$). While at 36 and 48 h, mRNA levels of *ACC*, *SREBP1* and *C/EBP α* still decreased with FGF21 treatment (Figs. 2e, 3d; $P > 0.05$), *PPAR γ* mRNA levels increased slightly ($P > 0.05$). Nevertheless, *FAS* expression was unaffected by FGF21 treatment throughout the experiment ($P > 0.05$).

Meanwhile, FGF21 treatment increased the levels of *ATGL*, *HSL*, *CPT-1* and *UCPI* mRNAs by 2–15 fold at 24 h (Fig. 3f; $P < 0.05$) and by 2–3.5 fold at 36 h (Fig. 3g; $P < 0.05$), but at 48 h only *ATGL* mRNA levels were significantly elevated (Fig. 3h; $P < 0.05$). The expression levels of *UCPI*, the most known lipolysis gene induced by FGF21, were decreased temporally.

goat	MGWDEAKFKHLGLWVPVLAVLLLLGTCRAHPIDSSPLLQFGGQVRQRYLYTDD	53
human	MDSDETGFEHSGLWVSVLAGLLLGACQAHPIPDSSPLLQFGGQVRQRYLYTDD	53
goat	AQETEAHLEIRADGTVVGAAARQSPESLLELKALKPGVIQIFGVKTSRFLCQGP	106
human	AQQTEAHLEIREDTVGGAADQSPESLLQLKALKPGVIQILGVKTSRFLCQRP	106
goat	DGKLYGSLHFDPKACSFRELLLEDGYNVYQSETLGLPLRLPPQRSSNRDPAPR	159
human	DGALYGSLHFDPEACSFRELLLEDGYNVYQSEAHGLPLHLPGNKSPHRDPAPR	159
goat	GPARFLPLPGLPPAPPDPPGILAPEPPDVGSSDPLSMVGPSYGRSPSYTS	209
human	GPARFLPLPGLPPAPPEPPGILAPQPPDVGSSDPLSMVGPSQGRSPSYAS	209

Fig. 2 Alignment of goat and human FGF21 amino acid sequences. The domain structure was analyzed by the online software SMART. Gray blocks represent the protein domain of

FGF21, red and blue blocks describe the receptor interaction binding sites and heparin binding sites of goat and human, respectively. (Color figure online)

Fig. 3 Effects of FGF21 on lipid droplet variation and gene expression pattern of adipogenesis and lipolysis in goat adipocytes. **a** Completely differentiated goat adipocytes were treated with FGF21. Untreated cells were stained with oil red O solution, visualized by a light microscope, and photographed after 24 h, 36 h and 48 h. **b** The sizes of lipid droplets were measured and quantified from the image of cells stained with oil red O. The staining of lipid droplets was analyzed using the

Image-Pro Plus system. Stained adipocytes were scanned using $\times 400$ objective magnification. Relative mRNA expression levels of *SREBP1*, *PPARγ*, *C/EBPα*, *ACC* and *FAS* in goat adipocytes treated with FGF21 and control shown at 24 h **c**, 36 h **d** and 48 h **e**. The mRNA levels of genes involved in lipolysis as determined by qRT-PCR at 24 h **f**, 36 h **g** and 48 h **h** (SEM, $n = 3$, $*p < 0.05$). (Color figure online)

Comparatively, FGF21 affected lipolysis genes more efficiently than lipogenesis genes since at 36 h the expression of the four genes involved in lipolysis still differed significantly ($P < 0.05$). Intriguingly, at 48 h *ATGL* mRNA was still consistently elevated, but accompanied by significantly downregulated expression of *SREBP1* and *C/EBPα*.

FGF21 induced mitochondria number and function in goat adipocyte

Generally, mitochondrial number increased throughout the investigation in cells treated with FGF21 (Fig. 4a), and simultaneously mtDNA copy number increased especially at 24 h (Fig. 4b–d).

Correspondingly, expression of *PGC1 α* , *NRF1*, and *TFAM*, which are involved in mitochondria biogenesis, were upregulated in adipocytes. Notably, *PGC1 α* exhibited significantly higher mRNA levels in treated adipocytes throughout the investigation ($P < 0.05$), whereas expression levels of *NRF1* and *TFAM* only significantly increased at 24 h and 36 h ($P < 0.05$; Fig. 4e–g). As for genes involved in mitochondrial fusion (*OPA1*, *Mfn1* and *Mfn2*) and fission (*FIS1*), FGF21 treatment increased mRNA levels of *OPA1* and

Mfn2 at 24 and 36 h, respectively ($P < 0.05$), but expression of *FIS1* was almost unaffected ($P > 0.05$; Fig. 4h). These results confirm that FGF21 mainly enhances mitochondrial biogenesis.

Discussion

FGF21 has been described as a hormone with a potential role in lipolysis in adipocytes. It has been

Fig. 4 Mitochondrial DNA copy number and mitochondrial-biogenesis-related gene expression in goat adipocytes treated with 75 nM FGF21. **a** Mitochondrial DNA copy number. The goat adipocytes treated with FGF21 and control cells were stained with Mito Tracker green at 24, 36 and 48 h, and then imaged using a fluorescence microscope ($\times 40$ magnification, $n = 3$). The cellular mtDNA content was assessed by qRT-PCR analysis with primers designed to target *COX2* and *GCG* as a

reference gene at 24 h **b**, 36 h **c** and 48 h **d** ($n = 3$). The ratio of *COX2* to *GCG* (Batista et al. 2014) reflects the number of mitochondria per adipocyte. The mRNA levels of *PGC1 α* , *NRF1*, and *TFAM* were analyzed using qRT-PCR and normalized to *Hsp-90*, *ALAS* and *HMBS* levels at 24 h **e**, 36 h **f** and 48 h **g** ($n = 3$, $*P < 0.05$). **h**: Treatment with FGF21, relative mRNA expression levels of *OPA1*, *Mfn1*, *Mfn2* and *FIS1* at 24h, 36h, and 48h

shown that *FGF21* transgenic mice cause lipolysis of mature adipocytes (Inagaki et al. 2007), and FGF21 protein stimulates lipolysis in cultured 3T3-L1 adipocytes (Chen et al. 2011). Our work found that FGF21 decreased the size of lipid droplets in goat adipocytes even at 48 h after treatment. This corresponded to elevated mRNA levels for *ATGL*, *HSL*, *CPT-1* and *UCP1* genes, which are critical in lipolysis (Dong et al. 2013; Walther and Farese 2012). These results confirm the potential role of FGF21 in adipocyte lipolysis.

Among the lipolysis genes investigated, *ATGL* maintained higher expression levels throughout the investigation. *ATGL* is the rate-limiting enzyme for lipolysis, specific for initiation of triacylglycerol hydrolysis (Ong et al. 2013), and highly expressed in adipose tissue (Wang et al. 2011). *ATGL*-Ad-infected 3T3-L1 cells and MEF (mouse embryonic fibroblasts) adipocytes have smaller lipid droplets (Miyoshi et al. 2008), which is consistent with the lipid droplet morphology observed in FGF21-treated goat adipocytes in our study. In addition, *ATGL*-mediated lipolysis controls the expression of *UCP1* by generating ligands or precursor ligands for nuclear receptors (Hondares et al. 2011). *ATGL* and *HSL* coordinately catabolize stored TG (Wang et al. 2013), as well. These data indicate that an increase in *ATGL* plays key role in lipolysis induced by FGF21.

Lipolysis genes were mostly affected by FGF21 treatment, and this behavior also closely relates to mitochondrial function. For example, *ATGL*-mediated lipolysis controls mitochondrial oxidative phosphorylation (Dimova et al. 2012); *CPT-1* accelerates the long-chain acetyl-CoA entry into mitochondria (Morris et al. 2012), which contain the primary sites for the tricarboxylic acid cycle and beta oxidation of fatty acids (Alaynick 2008). We found that FGF21 enhanced mitochondrial number, mtDNA copy number, and mRNA levels of mitochondrial biogenesis genes including *PGC1 α* , *TFAM* and *NRF1*. Specifically, *PGC1 α* was induced throughout all three stages investigated.

PGC1 α is a key factor of mitochondrial biogenesis. As a positive regulator of mitochondrial biogenesis and oxidative metabolism (Handschin and Spiegelman 2006), *PGC1 α* expression increased in brown adipocytes and muscle after cold exposure, hyperphagia, or adrenaline (Harms and Seale 2013; Sparks et al. 2005). A previous study demonstrated that FGF21 can

increase *PGC1 α* expression in human adipocytes (Chau et al. 2010). Our study also demonstrated that *PGC1 α* was upregulated in the presence of FGF21. We also observed that FGF21 enhanced the expression of *NRF1* and *TFAM*. *NRF1* is an intermediate transcription factor, which stimulates the synthesis of *TFAM* which is a final effector activating the duplication of mtDNA (Vina et al. 2009). Interestingly, overexpression of *PGC1 α* protein can stimulate genes including *NRF1* and *TFAM* (Doerks et al. 2002). Additionally, *UCP1* is located in downstream of *PGC1 α* (Fedorenko et al. 2012), and *UCP1* possesses mitochondrial oxidative capacity without concomitant synthesis of ATP (Flachs et al. 2013). The expression of *UCP1* mRNA indicates that at early stages of FGF21 stimulation (especially 24 h), lipid heat expenditure is critical in lipolysis. Furthermore, FGF21 treatment only partly elevated expression of genes associated with mitochondrial fusion (*OPA1* and *Mfn2*). These results imply that FGF21 elevates mitochondrial function mainly via mitochondrial biogenesis (including mtDNA copy number), in which *PGC1 α* may play key roles.

Simultaneously, expression of adipogenesis-related genes including *PPAR γ* , *SREBP1*, *C/EBP α* and *ACC* were downregulated dramatically by FGF21 treatment at 24 h. Adipogenesis is a process in which preadipocytes become adipocytes and begin lipid biosynthesis through conversion of acetyl-CoA into fatty acids and cholesterol (Shimano et al. 1999; Siersbaek et al. 2012). *ACC* is a key rate-limiting enzyme in de novo fatty acid synthesis (Postic and Girard 2008), while *PPAR γ* directly affects the accumulation of intracellular triglycerides (Grimaldi et al. 2010; Walczak and Tontonoz 2002) and cells lacking *PPAR γ* are unable to generate lipids even in the presence of an induction cocktail (Rosen et al. 1999). The dramatically downregulated *ACC* and *PPAR γ* mRNA levels in FGF21-treated adipocytes at 24 h suggest that de novo lipid synthesis may be restricted. This effect could be the main co-contributor to the obvious small lipid droplets in adipocytes with lipolysis genes.

Intriguingly, expression of *C/EBP α* and *SREBP1* dramatically decreased again at 48 h, after almost equal levels between control and treated adipocytes at 36 h. *C/EBP α* promotes transcription of genes specifically expressed in adipocytes (Karadeniz et al. 2011). *C/EBP α* and *PPAR γ* cross-regulate the transcriptional pathway of adipogenesis (Wu et al. 1999). *SREBP1*

deficiency in 3T3-L1 cells strongly inhibits adipocyte differentiation and suppresses the expression of *PPAR* γ , *C/EBP* α and *FAS* (Kim and Spiegelman 1996). Therefore, it is likely that *C/EBP* α and *SREBP1* are the main factors in a substantial network of suppressed lipogenesis factors induced by FGF21 treatment at later stages, but further studies are required to address this issue.

In conclusion, FGF21 decreased lipid droplet size in goat adipocytes as long as 48 h post-treatment and elevated mitochondrial function mainly via mitochondrial biogenesis (including mtDNA copy number). We also found that *ATGL* and *PGC1* α may play key roles in this process. Although FGF21 treatment induced lipolysis more effectively, suppression of lipogenesis, including de novo lipid synthesis, co-contributed to the smaller lipid droplets in adipocytes at 24 h, and at later stages (48 h) *C/EBP* α and *SREBP1*, the main lipogenesis factors, are suppressed by FGF21 treatment. Interestingly, *ATGL*-mediated lipolysis plays a critical role in generating ligands or precursor ligands for nuclear receptors, such as, *PPAR* α (Hondares et al. 2011). This suggests that lipolysis and lipogenesis are possibly coupled in a substantial network within FGF21 adipocytes, but further studies are required to address this issue.

Funding This work was supported by Sichuan province science and technology support program (2014NZ0077, 2015NZ0112).

Compliance with ethical standards

Conflict of interest All the authors stated no conflict of interest.

Ethical approval This work was approved by Institutional Animal Care and Use Ethics Committee of Sichuan Agriculture University and carried out in accordance with the “Guidelines for Experimental Animals” of the Ministry of Science and Technology (Beijing, PR China).

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